Lymphocyte subset numbers depend on the bacterial origin of sepsis

M. Holub[1,2], Z. Klučková[1], M. Helcl[3], J. Příhodov[3], R. Rokyta[4] and O. Beran[1]

[1]Charles University Prague, First Faculty of Medicine, 3rd Department of Infectious and Tropical Diseases, Czech Republic, [2]Wadsworth Center, Albany, NY, USA, [3]Department of Infectious Diseases, University Hospital Bulovka, Prague and [4]Medical ICU, University Hospital Pilsen, Pilsen, Czech Republic

Objective To determine the quantitative variances in peripheral blood lymphocyte subsets during sepsis, and their clinical significance.

Methods Peripheral blood lymphocyte subsets were enumerated in 32 non-surgical septic patients during the first 14 days of hospitalization; results from septic patients were compared with those from 34 healthy controls. Influences of the severity and the bacterial etiology of sepsis on changes in lymphocyte subsets were also assessed.

Results Significant decreases ($P < 0.05$) from normal values of $CD4^+$, $CD8^+$ and total T-lymphocytes were observed in septic patients, but the decline persisted only for $CD4^+$ T-lymphocytes and natural killer (NK) cells for 3 and 7 days, respectively. In addition, the numbers of $CD3^+/DR^+$ lymphocytes were significantly elevated on day 14. There were no correlations between these alterations and the severity of sepsis. Gram-positive sepsis ($n = 10$), which was mainly due to *Streptococcus pneumoniae and Staphylococcus aureus*, caused prolonged decreases in $CD4^+$, $CD8^+$ and total T-lymphocytes, and a reduction in NK cells, that lasted for $\geq$14 days. Conversely, patients with sepsis due to Gram-negative pathogens (*Neisseria meningitidis*, $n = 8$; enterobacteria, $n = 2$) achieved full recovery of the subsets within 3 days. Moreover, the patients with Gram-negative sepsis demonstrated a significant increase in B-lymphocytes, and a rise in the numbers of $CD3^+/DR^+$ and $CD4^+$ T-lymphocytes, which were more rapid than in patients with Gram-positive sepsis.

Conclusion Our results indicate that Gram-positive sepsis causes stronger suppression of peripheral blood lymphocyte subsets in comparison to sepsis due to Gram-negative pathogens.

Keywords Lymphocyte subsets, sepsis, etiology

Accepted 20 March 2002

Clin Microbiol Infect 2003; 9: 202–211

INTRODUCTION

Sepsis is an important microbial disease, with an annual incidence recently estimated to be more than 750 000 cases, contributing to 215 000 deaths per year in the USA [1]. The pathophysiology of sepsis is characterized by a systemic host response to microorganisms or their signal molecules (e.g. endotoxin) entering the bloodstream [2]. Sepsis-induced systemic inflammatory responses are controlled by contra-inflammatory mechanisms, which prevent organ injury due to exaggerated systemic inflammation. However, the contra-inflammatory responses may cause suppression of immune responses, leading to secondary infections [3]. Although complement and antibodies play an important role in the septic response, innate immunity and adaptive cell-mediated immunity (CMI) are probably the most suppressed immune functions during sepsis. The immunosuppressive effects of sepsis on innate immunity comprise reduced phagocytosis,

Corresponding author and reprint requests: M. Holub, Dept. of Infectious Diseases Budinove 2, Prague 8 CZ-18081, Czech Republic
E-mail: holubm@fnb.cz

decreased pro-inflammatory cytokine production and antigenic presentation by monocytes–macrophages, decreased adhesion, migration and oxidative burst of neutrophils, as well as reduced numbers of circulating natural killer (NK) cells [4–7]. Reductions in circulating $CD4^+$ T-lymphocytes and their shift to a Th2 phenotype characterize aspects of sepsis-induced suppression of adaptive CMI [8]. Interestingly, sepsis can also elicit profound depletion of B-lymphocytes as well as $CD4^+$ T-lymphocytes in secondary lymphoid organs, which may contribute to a decreased ability to combat infection [9]. The associations between complicated clinical course or unfavorable prognosis of septic patients with the decline of peripheral blood (PB) $CD4^+$ T-lymphocytes and activated T-lymphocytes ($CD3^+/DR^+$) were established in a majority of trauma victims or surgical patients with secondary sepsis [10,11]. A study performed by Williams et al. [12] on 31 non-surgical septic patients demonstrated no predictive value of T-lymphocyte abnormalities, although only four septic patients were examined longitudinally, and two patients with irreversible sepsis showed a persistent decrease in numbers of circulating T-lymphocytes.

The aim of the present study was to conduct longitudinal characterization of circulating lymphocyte subsets in non-surgical septic patients during the 2 weeks following onset of sepsis. The relationship of lymphocyte subset kinetics to the clinical course and bacterial origin of sepsis was also assessed.

PATIENTS AND METHODS

Patients

The prospective study was conducted in accordance with the Helsinki Declaration after obtaining institutional review board approval during the period from September 1998 to March 2000. Thirty-two consecutive septic patients (16 females and 16 males, mean age 32.9 years, range 10–78 years) admitted to the Department of Infectious Diseases of the University Hospital Bulovka, Prague, Czech Republic were enrolled into the study. Sepsis was diagnosed according to the ACCP/SCCM Consensus Conference as systemic inflammatory response syndrome (SIRS) with an infection [13]. All patients had to meet at least two of the following criteria for SIRS: (1) temperature above 38 °C or below 36 °C; (2) heart rate above 90 beats/min; (3) respiratory rate above 20 breaths/min or $PaCO_2 < 32$ mmHg; and (4) leukocytosis above 12×10^9/L or leukopenia below 4×10^9/L, or more than 10% band forms on a blood film, as well as the presence of infection. Infection was documented by the isolation of pathogenic bacteria from the blood or from other normally sterile sites. In addition, infection in patients without positive cultures was diagnosed clinically, and patients received empirical antibiotic treatment. Exclusion criteria included age below 10 years and above 80 years, treatment with immunomodulating therapy, HIV/AIDS, malignant disorder, surgery or trauma related to the presenting illness, and an interval between the onset of clinical symptoms of the disease and admission of more than 24 h. The control group consisted of 34 age- and gender-matched healthy persons.

Patients were followed for 14 days from the day of admission. The severity of the illness was assessed on admission according to the APACHE II score (Acute Physiologic and Chronic Health Evaluation) [14]. For the assessment of the clinical course of sepsis, we used the SOFA score (Sequential Organ Failure Assessment), C-reactive protein (CRP) plasma levels, and leukocytosis [2,15,16]. Two patients died during the study; the causes of their deaths were non-septic (malign arrhythmia and brain edema). An overview of patients' demographics, clinical diagnoses and laboratory findings is given in Table 1. In addition, microbiological follow-up was routinely performed for all patients admitted to the intensive care unit as well as patients with indwelling catheters hospitalized in standard wards. During the study, eight patients were diagnosed as having a nosocomial infection. There were 10 episodes of nosocomial infections (three cases of pneumonia, four urinary tract infections, and three cases of catheter-related sepsis) documented with an onset between 7 and 10 days after admission to the hospital.

Analysis of the influence of bacterial origin of sepsis utilized data from patients with an established definitive etiologic diagnosis of sepsis, which requires the isolation of microorganisms from the blood and/or from a local site of infection [2]. Bacterial pathogens were isolated from blood and/or cerebrospinal fluid (CSF), and patients with positive findings were divided into two groups: (1) Gram-positive sepsis due to *Streptococcus pneumoniae* ($n = 6$), *Staphylococcus aureus* ($n = 3$), or viridans streptococci ($n = 1$); and (2) Gram-negative

Table 1 Demographics and clinical characteristics of 32 septic patients

Patient	Age (years), sex	Clinical diagnosis	ICU stay	APACHE II at admission	Outcome	Blood or other site culture	Laboratory findings on admission			Hospitalization days
							White blood cells	CRP (mg/L)	T-cells/mm^3	
1	12, F	Sepsis	Yes	9	Recovered	*N. meningitidis* B, *A. baumani*	25.3	353	440	33
2	50, M	Pneumonia	Yes	12	Recovered	*Streptococcus pneumoniae*	15.5	465	200	32
3	24, M	Endocarditis	No	2	Recovered	*Staphylococcus aureus*	13.4	333	342	41
4	44, F	Purulent meningitis	Yes	8	Recovered	Negative	NA	258	NA	25
5	20, M	Endocarditis	No	1	Recovered	*Staphylococcus aureus*	NA	249	NA	34
6	41, F	Sepsis	Yes	5	Recovered	*P. mirabilis*	16.1	342	574	36
7	22, F	Purulent meningitis	Yes	2	Recovered	Negative	42.5	364	225	28
8	16, F	Purulent meningitis	Yes	1	Recovered	Negative	18.7	120	484	14
9	16, M	Purulent meningitis	Yes	12	Recovered	*Streptococcus hemolyticus;* CSF	22	163	390	34
10	15, F	Purulent meningitis	Yes	15	Recovered	*N. meningitidis* C	21	NA	140	19
11	71, F	Purulent meningitis	Yes	18	Died	*Streptococcus viridans*	23.9	NA	140	15
12	45, F	Purulent meningitis	Yes	2	Recovered	*Streptococcus pneumoniae*	14.7	268	130	15
13	54, M	Purulent meningitis	Yes	10	Died	Negative	30	312	540	13
14	26, M	Purulent meningitis	Yes	9	Recovered	Negative	23.2	148	390	14
15	14, M	Pneumonia	No	3	Recovered	*Streptococcus pneumoniae*	NA	312	NA	10
16	46, M	Purulent meningitis	Yes	5	Recovered	*E. coli*	22.5	279	180	44
17	23, M	Mediastinitis	Yes	3	Recovered	Negative	9.4	180	180	51
18	24, M	Purulent meningitis	Yes	2	Recovered	*N. meningitidis* C	21.2	517	190	25
19	17, F	Purulent meningitis	Yes	12	Recovered	*N. meningitidis* C	46.4	259	200	21
20	19, M	Purulent meningitis	Yes	3	Recovered	Negative	2.4	262	220	17
21	17, F	Purulent meningitis	Yes	1	Recovered	Negative	20.2	197	240	17
22	64, F	Purulent meningitis	Yes	10	Died	*Staphylococcus aureus*; CSF	19.9	432	440	50
23	27, F	Purulent meningitis	Yes	2	Recovered	*N. meningitidis* B	19.1	447	450	16
24	45, F	Purulent meningitis	Yes	6	Recovered	Negative	32.4	365	350	83
25	54, M	Purulent meningitis	Yes	23	Recovered	*Streptococcus pneumoniae*	16.4	559	110	58
26	78, F	Purulent meningitis	Yes	34	Recovered	*Listeria monocytogenes*	17	372	210	64
27	13, F	Pneumonia	No	6	Recovered	Negative	8.3	145	480	14
28	15, M	Purulent meningitis	No	7	Recovered	*N. meningitidis* B	3.7	235	50	10
29	51, F	Purulent meningitis	No	6	Recovered	*N. meningitidis*	26.9	139	340	28
30	10, M	Purulent meningitis	No	5	Recovered	*N. meningitidis* B	19.7	286	270	11
31	14, M	Sepsis	No	5	Recovered	Negative	22.5	365	162	17
32	65, M	Purulent meningitis	Yes	27	Recovered	*Streptococcus pneumoniae*	20.0	419	131	17

F, female; M, male; ICU, intensive care unit; NA, not available.

Table 2 Demographics, clinical characteristics and laboratory findings of patients with sepsis due to Gram-positive or Gram-negative pathogens

Characteristics	Gram-negative sepsis ($n = 10$)	Gram-positive sepsis ($n = 10$)	Student's *t*-test
Age, mean (years)	25.8 ± 4.8	42.3 ± 6.9	NS
Sex, female/male	6/4	3/7	NS[a]
Scores at admission			
APACHE II	15.7 ± 3.6	17.7 ± 3.0	NS
SOFA	4.1 ± 1.2	6.3 ± 1.7	NS
Outcome, recovered/died	10/0	8/2[b]	NS[a]
Hospitalization (days)	24.3 ± 4.8	30.6 ± 6.9	NS
ICU/standard ward (number of patients)	7/3	7/3	NS[a]
Laboratory findings on admission			
WBC (10^9 cells/L)	22.2 ± 3.4	18.0 ± 1.5	NS
CRP (mg/L)	317.4 ± 37.8	389.0 ± 53.9	NS

NS, non-significant (an α level ≤ 0.05 is considered statistically significant); WBC, white blood cells.
[a]Employed Fisher's exact test.
[b]Overall mortality (one patient died after the study).

sepsis caused by *Neisseria meningitidis* ($n = 8$), *Escherichia coli* ($n = 1$), or *Proteus mirabilis* ($n = 1$). An overview of the demographic and clinical data on these patients is presented in Table 2.

Laboratory methods

Blood samples were drawn in K-$_3$ EDTA Vacutainer tubes (Becton-Dickinson, San Jose, CA, USA) at baseline (day 0) in all patients enrolled into the study; additional samples were drawn at 3, 7 and 14 days after admission. White cell counts (Coulter STKS, Coulter Electronics Inc., Miami, FL, USA) and Wright-stained microscope differential leukocyte counts were performed to calculate the absolute number of leukocytes, lymphocytes and lymphocyte subsets per cubic millimeter of blood. Lymphocyte subsets in the blood were investigated using monoclonal antibodies and flow cytometry. PB lymphocyte subsets included total T-lymphocytes ($CD3^+$), $CD4^+$ T-lymphocytes ($CD3^+CD4^+$), $CD8^+$ T-lymphocytes ($CD3^+CD8^+$), total B-lymphocytes ($CD19^+$), NK cells ($CD3^-$ $CD16^+$ and $CD56^+$), and $CD3^+/DR^+$ lymphocytes ($CD3^+$/anti-HLA-DR^+). Immunophenotyping was done by two-color flow cytometric analysis (FACStrak or FACScalibur with Simulset software, Becton Dickinson Immunocytometry Systems, San Jose, CA, USA) using IMK-Lymphocyte Kit of monoclonal antibodies (containing Leukogate, Isotype Control, CD3/CD19, CD3/CD4, CD3/CD8, CD3/CD16+56 and CD3/anti-HLA-DR; Becton Dickinson, Heildelberg, Germany). The CD4/CD8 ratio was counted as the percentage of $CD4^+$ T-lymphocytes divided by the percentage of $CD8^+$ T-lymphocytes. This protocol is routinely used in the laboratory and undergoes a periodic evaluation in the international quality assurance scheme CEQUAL [17]. CRP plasma levels were measured with a nephelometer (Behring, Vienna, Austria) using set Latex CRP Mono (Behring) with a normal range between 0 and 8 mg/L.

Statistical analysis

Absolute numbers of leukocytes, lymphocytes and lymphocyte subsets were compared with the reference values obtained from the control group by one-way ANOVA and Dunn's method or Tukey's test. These statistical analyses were also used to test intergroup differences in regard to the bacterial origin of sepsis. Spearman correlation and linear regression were utilized for analysis of the influence of APACHE II, SOFA and CRP plasma levels on lymphocyte subset numbers. The α level of 0.05 was considered statistically significant. The data are presented, if not stated otherwise, as mean $\pm$ standard error (SE).

RESULTS

Kinetics of numbers of white blood cells and circulating lymphocytes

On admission, when compared with healthy controls, septic patients had significantly higher values of absolute numbers of blood leukocytes

(20.5 ± 1.5 versus 6.8 ± 0.3 cells/mm^3, $P < 0.05$). Although this level decreased after admission, significant leukocytosis persisted after 3 days (14.4 ± 1.5 cells/mm^3, $P < 0.05$) and 7 days (12.5 ± 1.2 cells/mm^3, $P < 0.05$) of hospitalization. In contrast, the absolute numbers of circulating lymphocytes were reduced significantly only at admission (0.9 ± 0.1 versus 2.0 ± 0.1 cells/mm^3, $P < 0.05$), and normalized thereafter. This recovery of circulating lymphocytes was delayed with Gram-positive sepsis. Three days after admission, the decline in lymphocyte counts still persisted (1229 ± 205 cells/mm^3, $P < 0.05$).

Kinetics of lymphocyte subset numbers (Figure 1)

The absolute numbers of total T-lymphocytes, when compared with values obtained from healthy controls, showed a marked drop from the norm (505 ± 80 versus 1435 ± 116 cells/mm^3, $P < 0.05$). As with the absolute lymphocyte count, the initial depression was followed by rapid recovery of circulating total T-lymphocytes. In patients with Gram-positive sepsis, reductions in the numbers of total T-lymphocytes were still apparent after 3 days of hospitalization (757 ± 142 cells/mm^3, $P < 0.05$).

A similar course was observed for the absolute numbers of CD4$^+$ T-lymphocytes. After the initial reduction (287 ± 28 versus 936 ± 62 cells/mm^3, $P < 0.05$), the CD4$^+$ T-lymphopenia was followed by a rapid and steady rise, but 3 days after admission there was still a significant decrease in the absolute numbers of CD4$^+$ T-lymphocytes (684 ± 96 cells/mm^3, $P < 0.05$). This persistent reduction was more evident with Gram-positive sepsis (520 ± 104 cells/mm^3, $P < 0.05$); patients with sepsis due to Gram-negative pathogens achieved full recovery of this subset by 3 days after admission. Moreover, the numbers of CD4$^+$ T-lymphocytes in the blood of septic patients with Gram-negative sepsis showed a trend for an increase above the norm. This rise was significant when compared with data obtained from patients with Gram-positive sepsis at day 7 (1359 ± 273 versus 672 ± 107 cells/mm^3, $P < 0.05$) and day 14 (1214 ± 200 versus 748 ± 121 cells/mm^3, $P < 0.05$) (Figure 2a).

The changes in circulating CD8$^+$ T-lymphocytes were parallel to the kinetics of changes in the absolute numbers of total T-lymphocytes. There was a significant decrease in the absolute numbers of CD8$^+$ T-lymphocytes after admission (251 ± 60 versus 499 ± 33 cells/mm^3, $P < 0.05$), and recovery was achieved by day 3. Gram-positive sepsis was responsible for a slower recovery of circulating CD8$^+$ T-lymphocytes: the significant fall in this subset still persisted after 3 days of hospitalization (166 ± 55 cells/mm^3, $P < 0.05$).

Circulating NK cells were also significantly reduced in septic patients (181 ± 35 versus 330 ± 30 cells/mm^3, $P < 0.05$). The absolute numbers of circulating NK cells did not rise over time and were significantly lower at 3 days (190 ± 20 cells/mm^3, $P < 0.05$) and 7 days (186 ± 18 cells/

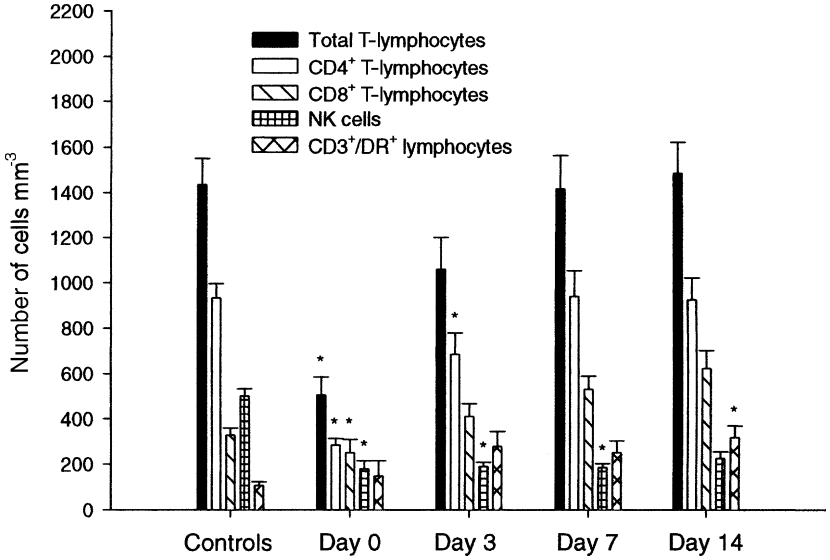

Figure 1 Kinetics of circulating total T-lymphocytes, CD4$^+$ T-lymphocytes, CD8$^+$ T-lymphocytes, CD3$^+$/DR$^+$ lymphocytes and NK cells in septic patients compared with values from healthy controls. *Statistically significant differences ($P < 0.05$) between septic patients and healthy controls.

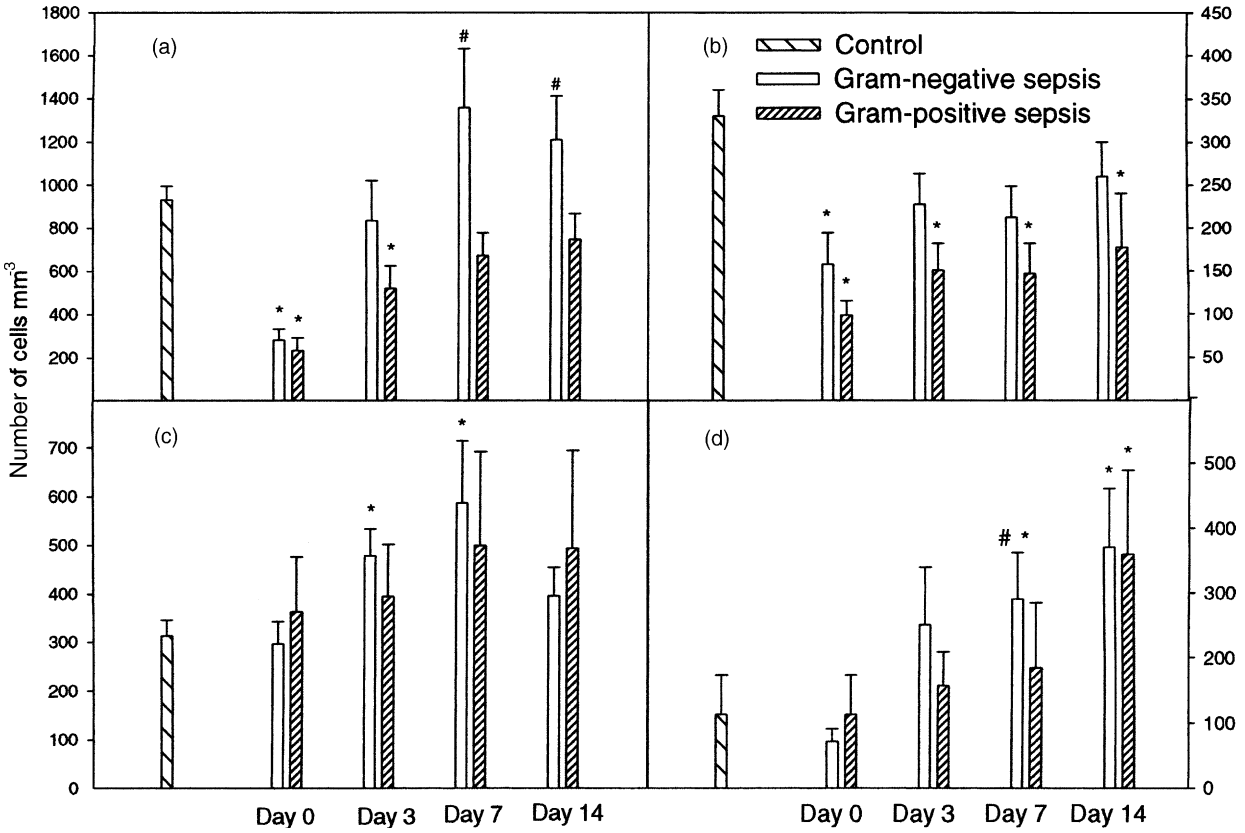

Figure 2 Kinetics of circulating $CD4^+$ T-lymphocytes (a), NK cells (b), B-lymphocytes (c) and $CD3^+/DR^+$ lymphocytes (d) in patients with Gram-positive sepsis and sepsis due to Gram-negative pathogens. *Significant differences ($P < 0.05$) from healthy controls; hash signs indicate significant difference ($P < 0.05$) between Gram-positive and Gram-negative sepsis.

mm^3, $P < 0.05$) after admission. Although their absolute numbers on day 14 were not statistically different from those of healthy controls, the trend for recovery was less apparent than for total T-lymphocytes. Interestingly, the numbers of circulating NK cells were significantly reduced on day 3 (150 ± 31 cells/mm^3, $P < 0.05$), day 7 (145 ± 35 cells/mm^3, $P < 0.05$) and day 14 (177 ± 63 cells/mm^3, $P < 0.05$) only with Gram-positive sepsis. Conversely, patients with sepsis caused by Gram-negative pathogens showed no significant differences in this subset after 3 days of hospitalization (Figure 2b).

The numbers of circulating B-lymphocytes in septic patients when compared with values obtained from healthy controls did not show significant changes (data not shown). Interestingly, sepsis due to Gram-negative pathogens was responsible for significant increases in the numbers of B-cells on day 3 (479 ± 55 versus 314 ± 32 cells/mm^3, $P < 0.05$) and day 7 (587 ± 128 cells/mm^3, $P < 0.05$). A similar trend was also observed with Gram-positive sepsis, but this increase was not significant (Figure 2c).

From the day of admission until 7 days after hospitalization, the absolute number of $CD3^+/DR^+$ lymphocytes was not significantly changed when compared to healthy controls: however, an increase was observed after 14 days of hospitalization (318 ± 54 versus 107 ± 17 cells/mm^3, $P < 0.05$). This increase was more rapid with sepsis due to Gram-negative pathogens. The numbers of circulating $CD3^+/DR^+$ lymphocytes were significantly higher than values obtained from patients with Gram-positive sepsis (291 ± 72 versus 184 ± 101 cells/mm^3, $P < 0.05$) or from healthy controls by day 7 (Figure 2d).

The CD4/CD8 ratios were not significantly changed, and there were no correlations between lymphocyte subset numbers with APACHE II and SOFA scores or CRP plasma levels of the septic patients or patients with an established etiologic

diagnosis of sepsis at any given point (data not shown). In addition, there was a significant negative correlation of the incidence of the nosocomial infections with reduced numbers of circulating B-lymphocytes on day 3 ($P = 0.04$, $r = -0.39$) and day 7 ($P = 0.02$, $r = -0.442$).

DISCUSSION

The most affected lymphocyte subsets in our group of septic patients were NK cells (Figure 1). These immune cells are important components of innate immunity; they do not require antigen-specific stimulation, and represent part of the first line of host defense [18]. The observed persistent reduction in the numbers of circulating NK cells might reflect their pronounced activation and intense trafficking into tissues during the initial stage of sepsis [19]. The protective effect of NK cells against infections comprises rapid production of interferon (IFN)-γ, contributing to intracellular killing of bacteria and the ability to destroy antibody-coated pathogens by a process called antibody-dependent cell-mediated cytotoxicity as well as perforin-mediated cytolysis or apoptosis (programmed cell death) of MHC class I negative cells [18,20–23]. Interestingly, the decrease in the numbers of NK cells observed during the study was influenced by the bacterial origin of sepsis. Patients with Gram-positive sepsis demonstrated a more persistent reduction in the numbers of circulating NK cells than that seen with Gram-negative sepsis (Figure 2b). This might indicate very intense activation of NK cells during severe Gram-positive infection, whose elimination usually requires highly organized host responses, whereas many Gram-negative pathogens are efficiently destroyed by complement and antibodies [24]. After activation, NK cells can be eliminated by apoptosis to prevent excessive production of IFN-γ, which may, in turn, cause an exaggerated activation of monocytes and massive release of pro-inflammatory cytokines such as interleukin-1β and tumor necrosis factor (TNF)-α [25,26]. On the other hand, excessive apoptosis of NK cells also may lead to persistent reduction in their circulating numbers, which has been shown to be an important sign of immunosuppression predisposing trauma victims to secondary bacterial infections [27].

Significant reductions in circulating T-lymphocytes and their respective subsets observed during the acute phase of sepsis were transient (Figure 1) and independent of the severity of the illness. Although the loss of lymphocytes in sepsis is often considered to be due to apoptosis [9], rapid recovery of total, $CD4^+$ and $CD8^+$ T-lymphocytes could also indicate their intense trafficking between tissues and the lymphatic system during the acute phase of the illness [28]. Reductions in circulating $CD4^+$ and $CD8^+$ T-lymphocytes also could be elicited by release of high amounts of endogenous corticosteroids or TNF-α, which are raised due to a stress response in the early stage of severe bacterial infections and sepsis [29–32]. Interestingly, in animal experiments, it has been documented that changes in lymphocyte subset distribution are reversed swiftly after the cessation of stress [16]. In our study, broad-spectrum antibiotics suppressed bacterial infection in all septic patients by day 3, as was documented by the significant decrease in CRP plasma level (data not shown), which is considered a good indicator of the resolution of sepsis [33]. Therefore, rapid normalization of the absolute numbers of circulating $CD4^+$ and $CD8^+$ T-lymphocytes in patients enrolled in the study is, in our opinion, caused by suppression of bacterial growth and cessation of bacterial stress.

Interestingly, the patterns of the recovery of circulating total T-lymphocytes and their respective subsets were dependent upon the bacterial etiology of sepsis. Rapid normalization of the numbers of T-lymphocytes and their subsets in the blood, and especially the trend for the increase in the numbers of PB $CD4^+$ T-lymphocytes above the norm, were characteristic for sepsis caused by Gram-negative pathogens (Figure 2a). *N. menigitidis* was the prevailing etiologic agent of the Gram-negative sepsis, and some meningococcal antigens can stimulate $CD4^+$ T-lymphocyte proliferation [34]. However, two patients had Gram-negative sepsis due to enterobacteria (*P. mirabilis and E. coli*), and they showed similar trends as patients with meningococcal sepsis, with high numbers of PB $CD4^+$ T-lymphocytes on days 7 and 14. The primary role in the pathogenesis of Gram-negative sepsis has been assigned to endotoxin [24]. Endotoxin can synergize with antigenic peptides and increase proliferation of T-lymphocytes [35], which may be followed by their accumulation in the blood. Although *S. aureus* and *S. pneumoniae* (the prevailing etiologic agents in patients with Gram-positive sepsis) have been

shown to cause early polyclonal activation of T-lymphocytes [36,37], the increase in the numbers of circulating $CD4^+$ T-lymphocytes was significantly less than that observed with Gram-negative sepsis. This could be due to activation-induced cell death (AICD) of those activated T-lymphocytes in the tissues [38]. Thus, AICD might be an important mechanism responsible for slow recovery of circulating $CD4^+$ T-lymphocytes in patients with Gram-positive sepsis.

The marked increase in the numbers of circulating B-lymphocytes with sepsis caused by Gram-negative pathogens and the trend for their numbers to rise above the norm in patients with Gram-positive sepsis on day 3 and day 7 (Figure 2c) may represent the transition from innate to more specific adaptive immune responses. The increase in the numbers of circulating B-lymphocytes in patients with Gram-negative sepsis was similar to the increase in $CD4^+$ T-lymphocytes, and this finding was apparent regardless of the Gram-negative etiology of the illness. Significant accumulation of B-lymphocytes in the blood of patients recovering from Gram-negative sepsis might represent intensive B-lymphocyte proliferation due to enhanced T–B-lymphocyte cooperation [18]. Conversely, *S. aureus* and *S. pneumoniae* could be directly responsible for the trend in the increase of PB B-lymphocyte numbers above the norm observed in patients recovering from Gram-positive sepsis, as both pathogens were documented as being polyclonal activators of B-lymphocytes [39]. As was shown in an animal model of sepsis elicited by parasitic infection, the proliferation of B-lymphocytes takes place in the spleen and may be subsequently followed by their appearance in the blood [40]. In addition, significant correlation of reduced numbers of B-lymphocytes on days 3 and 7 with the incidence of nosocomial infection in our patients might suggest the importance of this subset as a clinical marker of sepsis-induced immunosuppression. However, bacterial etiology (data not shown) and the sites of nosocomial infections were considerably different, which complicates evaluation of the finding in relatively small numbers of patients.

Non-surgical septic patients, when compared to control subjects, showed no significant change in numbers of circulating $CD3^+/DR^+$ lymphocytes [41], which is compatible with our observations during the first week of non-surgical sepsis. However, the numbers of circulating $CD3^+/DR^+$ lymphocytes significantly rose in our patients on day 14 (Figure 1), probably reflecting late lymphocyte activation and development of specific immune responses [42]. Interestingly, a slower rise in the numbers of circulating $CD3^+/DR^+$ lymphocytes, which was observed with Gram-positive sepsis when compared to a more rapid increase in this subset in patients with Gram-negative sepsis (Figure 2d), may indicate a delay in the adaptive immune reactions during severe Gram-positive infection. In addition, two patients who died during the study demonstrated low numbers of PB $CD3^+/DR^+$ lymphocytes and NK cells before death (data not shown). Both persons succumbed to non-septic complications after resolution of sepsis, and the numbers of $CD4^+$ and $CD8^+$ T-lymphocytes as well as B-lymphocytes in the blood rose over time. The decrease in the numbers of PB $CD3^+/DR^+$ lymphocytes and NK cells observed in the non-survivors might suggest a high sensitivity of these subsets to stress conditions.

In conclusion, our results indicate that sepsis-induced decreases in lymphocyte subsets in the blood are independent of the severity but not the bacterial etiology of the illness. However, whether these differences only reflect specific host responses to certain pathogens or also play a role in the pathophysiology of sepsis needs to be clarified.

ACKNOWLEDGMENTS

This study was supported by the Grant Agency of the Ministry of Health of the Czech Republic (grant no. 4972-3), and partly also by grant no. 3D43TW00915 from the Fogarty International Center, National Institutes of Health. The authors would like to thank Dr David A. Lawrence (Wadsworth Center, Albany, NY, USA) for reviewing the manuscript, and Dr Pavel Dřevínek (University Hospital Motol, Prague, Czech Republic) for help with the data analysis.

REFERENCES

1. Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinski MR. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. *Crit Care Med* 2001; 29: 1303–10.
2. Munford RS. Sepsis and septic shock. In: Fauci AS, Braunwald E, Isselbacher KJ *et al*., eds. *Harrison's*

principles of internal medicine, 14th edn. New York: McGraw-Hill, 1998: 776–80.
3. Volk HD, Reinke P, Docke WD. Clinical aspects: from systemic inflammation to 'immunoparalysis'. *Chem Immunol* 2000; 74: 162–77.
4. Reddy RC, Chen GH, Tekchandani PK, Standiford TJ. Sepsis-induced immunosuppression. *Immunol Res* 2001; 24: 273–87.
5. Volk HD, Reinke P, Krausch D *et al.* Monocyte deactivation—rationale for a new therapeutic strategy in sepsis. *Intensive Care Med* 1996; 22(suppl 4): S74–81.
6. Wenish C, Parschalk P, Hasenhundl M, Griesmacher A, Graninger W. Polymorphonuclear leykocyte dysregulation in patients with gram-negative bacteraemia assessed by flow cytometry. *Eur J Clin Invest* 1995; 25: 418–24.
7. Holub M, Klućková Z, Beneda B, Hužička I, Hobstová J, Lobovská A. Changes in lymphocyte subpopulations and $CD3^+/DR^+$ expression in sepsis. *Clin Microbiol Infect* 2000; 6: 657–60.
8. Ferguson NR, Galley HF, Webster NR. T helper cell subset ratios in patients with severe sepsis. *Intensive Care Med* 1999; 25: 106–9.
9. Hotchkiss RS, Tinsley KW, Swanson PE *et al.* Sepsis-induced apoptosis causes progressive profound depletion of B and $CD4^+$ T-lymphocytes in human. *J Immunol* 2001; 166: 6952–63.
10. Cheadle WG, Pemberton MR, Robinson D, Livingston DH, Rodriguez JL, Pokl HC. Lymphocyte subsets responses to trauma and sepsis. *J Trauma* 1993; 35: 844–9.
11. Wakefield CH, Carey PD, Foulds S, Monson JR, Guillou PJ. Changes in major histocompatibility complex class II expression in monocytes and T cells of patients developing infection after surgery. *Br J Surg* 1993; 80: 205–9.
12. Williams RC, Kostler FT, Kilpatrick KA. Alteration in lymphocyte cell surface markers during various human infections. *Am J Med* 1983; 75: 807–16.
13. Members of the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine consensus conference: definition of sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Crit Care Med* 1992; 20: 864–74.
14. Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. *Crit Care Med* 1985; 13: 374–83.
15. Moreno R, Vincent JL, Matos R *et al.* The use of maximum SOFA score to quantify organ dysfunction/failure in intensive care. Results of a prospective, multicentre study. Working group on sepsis related problems of the ESICM. *Intensive Care Med* 1999; 25: 686–96.
16. Yentis SM, Soni I, Sheldon J. C-reactive protein as an indicator of resolution of sepsis in the intensive care unit. *Intensive Care Med* 1995; 21: 602–5.
17. Veselϖ R, Barths J, Vanlangendonck F, Hannet I, Struss K. Initial results of central European immunophenotyping quality control program. *Cytometry* 1996; 26: 108–12.
18. Goldsby RA, Kindt TJ, Osborne BA, eds. *Kuby immunology*, 4th edn. New York: W. H. Freeman and Co.
19. Toft P, Dagnaes-Hansen F, Tønnesen E, Basse PM. The effect of surgical stress and endotoxin-induced sepsis on the NK-cell activity, distribution and pulmonary clearance of YAC-1 and melanoma cells. *APMIS* 1999; 107: 359–64.
20. Bancroft GJ. The role of natural killer cells in innate resistance to infection. *Curr Opin Immunol* 1993; 5: 503–10.
21. Johnson RM, Brown EJ. Cell-mediated immunity in host defense against infectious diseases. In: Mandell GL, Bennett JE, Dolin R, eds. *Principles and practice of infectious diseases*, 5th edn. Philadelphia: Churchill Livingstone, 2000: 112–46.
22. Docke WD, Randow F, Syrbe U *et al.* Monocyte deactivation in septic patients: restoration by IFN-γ treatment. *Nat Med* 1997; 3: 678–81.
23. Bogdan C, Rollinghoff M, Diefenbach A. The role of nitric oxide in innate immunity. *Immunol Rev* 2000; 173: 17–26.
24. Opal SM, Cohen J. Clinical gram-positive sepsis: does it fundamentally differ from Gram-negative sepsis? *Crit Care Med* 1999; 27: 1608–16.
25. Hotchkiss RS, Swanson PE, Freeman BD *et al.* Apoptotic cell death in patients with sepsis, shock, and multiple organ dysfunction. *Crit Care Med* 1999; 27: 1230–51.
26. Medzhitov R, Janeway ChA. Innate immunity: impact on the adaptive immune response. *Curr Opin Immunol* 1997; 9: 4–9.
27. Ditschowski M, Kreuzfelder E, Majetschak M, Obertacke U, Schade VF, Grosse-Wilde H. Reduced B cell HLA-DR expression and natural killer cell count in patients prone to sepsis after injury. *Eur J Surg* 1999; 165: 1129–33.
28. Lemaire LCJM, Van Deventer SJH, Van Lanschot JJB, Meenan J, Gouma DJ. Phenotypical characterization of cells in the thoracic duct of patients with and without systemic inflammatory response syndrome and multiple organ failure. Scand J Immunol 1998; 47: 69–75.
29. Monig H, Arendt T, Meyer M, Kloehn S, Bewig B. Activation of the hypothalamo-pituitary-adrenal axis in response to septic or non-septic diseases—implication for the euthyroid sick syndrome. *Intensive Care Med* 1999; 25: 1402–6.
30. Dhabhar FS, Miller AH, McEwen BS, Spencer RL. Stress-induced changes in blood leukocyte distribution. *J Immunol* 1996; 157: 1638–44.

31. Payen D, Faivre V, Lukaszewicz AC, Losser MR. Assessment of immunological status in the critically ill. *Minerva Anestesiol* 2000; 66: 351–7.
32. Dongsoo K, Reilly A, Lawrence DA. Relationship between IFNγ, IL-6, corticosterone, and *Listeria monocytogenes* pathogenesis in BALB/c mice. *Cell Immunol* 2001; 207: 13–18.
33. Dhabhar FS, Miller AH, McEwen BS, Spencer RL. Effects of stress on immune cell distribution. Dynamics and hormonal mechanisms. *J Immunol* 1995; 154: 5511–27.
34. Kizil G, Todd I, Atta M, Borriello SP, Ait-Tahar K, Ala'Aldeen DA. Identification and characterization of TspA, a major CD4 (+) T-cell- and B-cell-stimulating Neisseria-specific antigen. *Infect Immun* 1999; 67: 3533–41.
35. Goodier MR, Londei M. Low concentration of lipopolysaccharide synergize with peptides to augment T-cell proliferation and can prevent the induction of non-responsiveness by CTLA4-Ig. *Immunology* 2001; 102: 15–23.
36. Miyamoto YJ, Wann ER, Fowler T, Duffield E, Hook M, McIntyre BW. Fibronectin binding protein A of *Staphylococcus aureus* can mediate human T lymphocyte adhesion and coactivation. *J Immunol* 2001; 166: 129–38.
37. Arva E, Andersson B. Kinetics of cytokine release and expression of lymphocyte cell-surface activation markers after *in vitro* stimulation of human peripheral blood mononuclear cells with *Streptococcus pneumoniae*. *Scand J Immunol* 1999; 49: 237–43.
38. Janssen O, Sanzenbacher R, Kabelitz D. Regulation of activation-induced cell death of mature T-lymphocyte populations. *Cell Tissue Res* 2000; 301: 85–99.
39. Abdelnour A, Tarkowski A. Polyclonal B-cell activation by an arthritogenic *Staphylococcus aureus* strain: contribution of T-cells and monokines. *Cell Immunol* 1993; 147: 279–93.
40. Helmby H, Jonsson G, Troye-Blomberg M. Cellular changes and apoptosis in the spleens and peripheral blood of mice infected with blood-stage *Plasmodium chabaudi chabaudi* AS. *Infect Immun* 2000; 68: 6087–9.
41. Lin RY, Astiz ME, Saxon JC, Rackow EC. Altered leukocyte immunophenotypes in septic shock. Studies of HLA-DR, CD11b, CD14, and IL-2R expression. *Chest* 1993; 104: 847–53.
42. Benjamini E, Sunshine G, Leskowitz S, eds. *Immunology, a short course*, 3rd edn. New York: Wiley-Liss, 1996: 199–202.